Metal Complexes of Glutamic Acid-γ-hydroxamic acid (Glu-γ-ha) (*N*-Hydroxyglutamine) in Aqueous Solution

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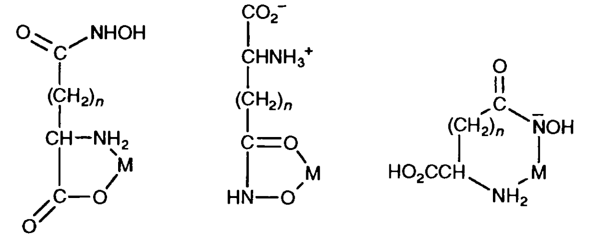
Stability constants and assumptions concerning the bonding mode are summarized for the complexes formed in aqueous solution in nickel(II)-, copper(II)-, zinc(II)- and iron(III)-L-glutamic acid-γ-hydroxamic acid (Glu-γ-ha) systems and nickel(II)-, copper(II)- and zinc(II)-acetohydroxamic acid-α-alanine ternary systems as models. Mononuclear species have been found with bidentate co-ordination of the HA^- (monoprotonated) and tridentate co-ordination of the A^{2-} (deprotonated) form of Glu-γ-ha under the conditions employed. Co-ordination of the hydroxamate and carboxylate oxygens is proposed in the case of iron(III). Complexes with different bonding modes existing in equilibria are formed with nickel(II) and copper(II), while preference for co-ordination *via* hydroxamate oxygens and amino-N is found with zinc(II).

The biological importance of hydroxamic acids is well established as well as their strong chelating abilities (*O,O*-chelate), especially towards hard metal ions.[1,2] The complexation behaviour of aminohydroxamic acids has been reviewed recently.[3] In the case of α-aminohydroxamic acids [*e.g.* glycinehydroxamic acid (gha), $NH_2CH_2CONHOH$], the (*N,N*) chelate is preferred over the (*O,O*) chelate with borderline metal ions as first proved by a crystal structure of the nickel(II) complex of gha[4] formed over the whole complexation pH range and by Cu^{II} only at pH values greater than 6.[5]

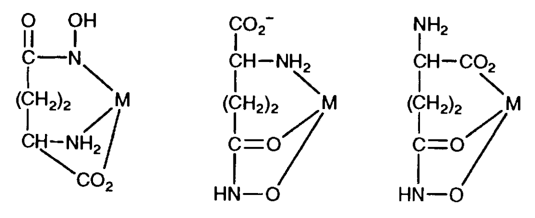
For β-aminohydroxamic acids (*e.g.* β-alaninehydroxamic acid) six-membered (*N,N*) chelates are formed by Ni^{II} whereas Cu^{II} forms polynuclear species containing both six- (*N,N*) and five-membered (*O,O*) chelates.[6]

The situation is even more complicated in the case of the β- and γ-hydroxamic acid derivatives of aspartic acid and glutamic acid (Asp-β-ha) (*n* = 1, Scheme 1) and (Glu-γ-ha) (*n* = 2, Scheme 1). Both acids may co-ordinate as bidentate ligands in three ways: (*i*) as an amino acid (*N,O* chelation *via* the amino-N and carboxylate-O), (*ii*) as a hydroxamate (*O,O* chelation) or (*iii*) with (*N,N*) chelation leading to six- and seven-membered rings for Asp-β-ha and Glu-γ-ha, respectively (see Scheme 1). Recent studies of the complexation of Asp-β-ha with Cu^{II} show the formation of a very stable polynuclear species probably involving all the above three types of chelation.[7] Finally, Glu-γ-ha and Asp-β-ha may act as tridentate ligands involving (*O,N,N*) and (*O,O,O*) co-ordination as shown in Scheme 2. In the case of Ni^{II}, Asp-β-ha probably co-ordinates in the (*O,N,N*) mode whilst with Fe^{III} it probably co-ordinates through the (*O,O,O*) mode.[7]

In the present paper, we report a study of the complexation behaviour of Glu-γ-ha [$H_3A^+ = HO_2C\text{-}CH(\overset{+}{N}H_3)(CH_2)_2\text{-}CONHOH$] with Cu^{II}, Ni^{II}, Zn^{II} and Fe^{III} by pH-metric, UV/VIS spectrophotometric, EPR, IR and ^1H NMR methods and comparison of the results with analogous studies of metal ion–Asp-β-ha binary and metal ion–acetohydroxamic acid (aha)-α-alanine (α-Ala) ternary systems as models.


(*a*) (*N,O*) (*b*) (*O,O*) (*c*)(*N,N*)

Scheme 1 Possibilities for the formation of five-membered chelates with the HA^- form of Glu-γ-ha (*n* = 2) and Asp-β-ha (*n* = 1); (*a*) amino acid type chelate, (*b*) hydroxamate type chelate. The alternative six- (*n* = 1) or seven- (*n* = 2) membered chelating mode is shown in (*c*)


(*a*) (*b*) (*c*)

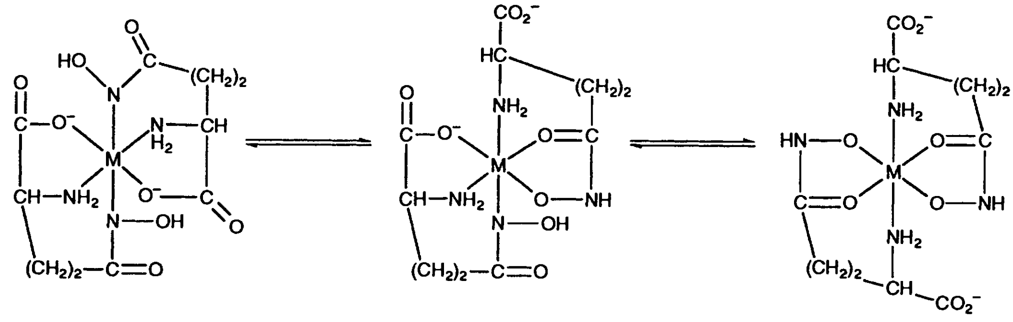
Scheme 2 Possible tridentate co-ordination modes with Glu-γ-ha; (*a*) (*O,N,N*) co-ordination, (*b*) (*O,O,N*) co-ordination or (*c*) (*O,O,O*) co-ordination

Experimental

The reagents Glu-γ-ha, Asp-β-ha, aha (Sigma) and α-Ala (Reanal) were used as ligands. Their purity and the exact concentration of their stock solutions were determined by Gran's method.[8]

The pH-metric and UV/VIS measurements were carried out in H_2O; EPR measurements in H_2O–MeOH (95:5) and ^1H NMR and IR measurements in D_2O (Aldrich Chemie).

Metal-ion stock solutions were prepared from copper(II) chloride dihydrate (Reanal), nickel(II) chloride hexahydrate (M&B) (dried in an oven at 175 °C for IR measurements), zinc(II) oxide (Hopkins and Williams) and iron(III) chloride hexahydrate (BDH) as described previously.[9]



Scheme 3 Most probable bonding modes of MA_2 existing in dynamic equilibrium in the Ni^{II}– and Cu^{II}–Glu-γ-ha systems

The pH-metric titrations for the various metal ion–Glu-γ-ha systems were carried out at five or six different metal ion : ligand ratios in the range 1 : 1–1 : 6. The concentration of the ligand was varied within the range 1×10^{-2}–4×10^{-3} mol dm^{-3}. The titrations for metal ion–aha–α-Ala ternary systems were performed at 1 : 1 : 1, 1 : 2 : 1, 1 : 1 : 2 and 1 : 2 : 2 ratios with the components in 6×10^{-3} and 3×10^{-3} mol dm^{-3} concentrations. All measurements were made at 25 °C at an ionic strength of 0.2 mol dm^{-3} (KCl) using a Radiometer pHM 84 pH-meter with a GK 2301 combined electrode. The electrode system was calibrated by the method of Irving *et al.*[10] so that the pH-meter readings could be converted into hydrogen ion concentrations. The calculations were performed with the aid of the PSEQUAD computer program.[11]

The absorption spectra for copper(II)–Glu-γ-ha were recorded on a Varian DMS100 double-beam recording spectrophotometer over the range 350–800 nm at 1 : 2 and 1 : 4 metal : ligand ratios [the concentration of copper(II) ion was 2×10^{-3} mol dm^{-3}]. The spectra for nickel(II)– and iron(III)–Glu-γ-ha and for nickel(II)–aha as a model system were recorded on a Perkin Elmer Lambda 6 double-beam UV/VIS spectrophotometer. In the case of nickel(II)-containing systems, a 1 : 4 metal : ligand ratio was used and the concentration of nickel(II) ion was 2×10^{-2} mol dm^{-3}. The iron(III)–Glu-γ-ha system was studied using 1 : 20 and 1 : 10 metal : ligand ratios at 1×10^{-3} mol dm^{-3} iron(III) concentration. The former systems were studied over the range 300–800 nm, the latter one in the 300–600 nm region.

The EPR measurements on copper(II)–Glu-γ-ha were made at liquid-nitrogen temperature on a JES-ME-3F spectrometer.

A JEOL JNM-GX270 FTNMR spectrometer was used to record the ^1H NMR spectra for iron(III)–, nickel(II)– and zinc(II)–Glu-γ-ha. The concentration of the ligand was 5×10^{-2} mol dm^{-3} in all samples and 3-trimethylsilylpropanesulfonic acid was used as internal reference. Metal : ligand ratios of 1 : 2 or 1 : 4 were used for the zinc(II)–Glu-γ-ha system to measure the chemical shifts; 1 : 100 and 1 : 30 ratios were used to investigate the line broadening effects of iron(III) and nickel(II), respectively.

The IR measurements were carried out on a Perkin Elmer 1710 FTIR spectrometer at 5×10^{-2} mol dm^{-3} ligand concentrations using CaF_2 cells. For storage of spectra, the software Perkin Elmer 1700 Data Station was used.

Results and Discussion

As discussed above, Glu-γ-ha may co-ordinate as a bidentate ligand in three modes of chelation: an amino-acid type (*N,O*), a hydroxamate type (*O,O*) or a (*N,N*) type and as a tridentate ligand in a further three modes (*O,N,N*), (*O,O,N*) and (*O,O,O*); the possibility of a mixed type chelation occurring through a dynamic equilibrium of a number of the above modes must also be considered (see Scheme 3). To clarify the problem model ternary systems were studied; thus the two five-membered chelate forming moieties of Glu-γ-ha [Scheme 1(*a*) and (*b*)] were modelled by α-alanine (α-Ala) and acetohydroxamic acid (aha), respectively. The dissociation constants of aha and α-Ala and the stability constants of their binary and ternary complexes formed with copper(II), nickel(II) and zinc(II) are listed in Table 1 and the related species distribution curves formed in the copper(II)–, nickel(II)– and zinc(II)–aha-α-Ala systems at 1 : 2 : 2 ratios are plotted in Figs. 1–3.

As seen from Table 1, the ternary complexes formed with copper(II) and nickel(II) have enhanced stability ($\Delta\log \beta > 0$) which is well illustrated by Figs. 1 and 2. With zinc(II), however, (*O,O*) co-ordination is somewhat preferred.

In the above equilibrium studies the dissociation constants of Glu-γ-ha were determined first. The totally protonated Glu-γ-ha (H_3A^+) can release three protons in the measurable pH region (from $-CO_2H$, $-NH_3^+$ and –CONHOH groups). The dissociation macroconstants (p*K*) are: 2.21(1), 8.55(1) and 9.50(1). The first value (pK_1) can be assigned to the dissociation of the $-CO_2H$ group but the other two processes overlap each other[12] which means that the pK_2 and pK_3 macroconstants are not characteristic of the real acidity of the $-NH_3^+$ and –CONHOH groups. Microscopic dissociation constants should clarify the acid–base properties of Glu-γ-ha.

Metal Complexes.—The models yielding the best fittings between the measured and calculated titration curves together with the refined stability constants are summarized in Table 2 which also contains the data on the metal ion–Asp-β-ha systems published in ref. 7.

As Table 2 shows, the pH-metric data can be fitted closely with the same model in all systems. This model contains a number of protonated complexes but not a 1 : 3 species nor any polynuclear ones. To obtain further information about the bonding modes in the complexes formed in the different systems, UV/VIS, ^1H NMR, IR and EPR measurements were made.

The results for the iron(III)–Glu-γ-ha system are in good agreement with those for iron(III)–Asp-β-ha[7] in which tridentate co-ordination *via* hydroxamate and carboxylate oxygens was found [Scheme 2(*c*)]. For example, the λ_{max} value in the visible spectra are at *ca.* 420 nm at pH *ca.* 4.5 and the line-broadening effects in the ^1H NMR spectra are the same for all carbon protons, as can be seen from Fig. 4, supporting (*O,O,O*) co-ordination from both ends of Glu-γ-ha. The hydroxamate oxygens and the carboxylate group are therefore the main co-ordinating donors. The amino group must contain the dissociable protons in the protonated species. Their deprotonation starts above pH 4 and may overlap with deprotonation of co-ordinated water.

Comparison of the pH-metric results for copper(II)–Glu-γ-ha with those for copper(II)–Asp-β-ha (Table 2) shows a marked difference. Only mononuclear species are found in the former system in contrast to a stable polynuclear complex in the latter. On the other hand, comparing the results for the nickel(II)– and

Table 1 Refined stability constants (log β) and $\Delta\log\beta_{1110}$ values* for complexes formed in copper(II)–, nickel(II)– and zinc(II)–aha-α-Ala binary and ternary systems [I = 0.2 mol dm^{-3} (KCl), T = 298 K]

Ligand	Species $M_pA_qB_rH_s$	Cu^{II}	Ni^{II}	Zn^{II}
aha	0101	9.26(1)		
	1100	7.89(1)	5.15(1)	5.18(2)
	110 − 1		−4.35(3)	−3.40(2)
	1200	14.06(2)	9.18(1)	9.45(3)
	120 − 1	4.44(4)		
	1300			
α-Ala	0011	9.68(1)		
	0012	12.03(2)		
	1010	8.04(1)	5.32(2)	4.56(2)
	101 − 1			−3.60(4)
	1020	14.73(1)	9.74(2)	8.51(2)
	102 − 1		−1.79(3)	−0.15(5)
	1030		12.80(5)	
aha-α-Ala	1110	14.93(3)	9.87(4)	9.28(2)
	1210		12.75(5)	11.98(5)
	1120		13.27(3)	
	$\Delta\log\beta_{1110}$	+0.23	+0.11	0.0

* $\Delta\log\beta_{1110} = \frac{1}{2}(\log\beta_{1200} + \log\beta_{1020} + \log 4)$.

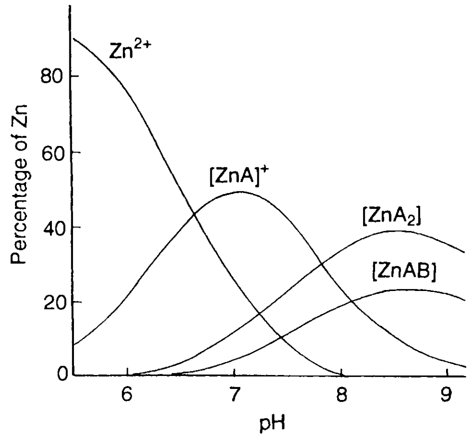


Fig. 1 Concentration distribution curves of complexes formed in the zinc(II)–aha-α-Ala system at 1:2:2 ratio. $c_{Zn^{II}}$ = 3 × 10^{-3} mol dm^{-3}

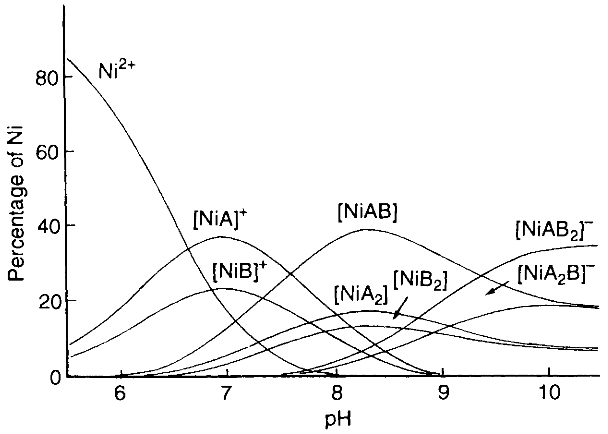


Fig. 2 Concentration distribution curves of complexes formed in the nickel(II)–aha-α-Ala system at 1:2:2 ratio, $c_{Ni^{II}}$ = 3 × 10^{-3} mol dm^{-3}

zinc(II)–Glu-γ-ha systems with the corresponding ones for Asp-β-ha, no significant differences are observed other than some differences in the stability constants which can be explained if we take into account that the amino and hydroxamate ends are more separated from each other in Glu-γ-ha than in Asp-β-ha.

Table 2 Refined stability constants (log β) of complexes formed in metal ion–Glu-γ-ha and –Asp-β-ha systems

Ligand	Species $M_pA_qH_r$	Cu^{II}	Ni^{II}	Zn^{II}	Fe^{III}
Asp-β-ha*	011	9.37(1)			
	012	17.52(1)			
	013	19.70(1)			
	111	16.41(16)	14.45(3)	13.91(2)	18.82(5)
	110	13.24(5)	8.38(2)	7.44(1)	
	212				36.35(5)
	211		21.74(6)	20.30(3)	31.63(6)
	210		14.42(3)	11.72(3)	24.25(7)
	21 − 1		4.56(10)	2.11(3)	15.32(7)
	44 − 2	52.82(16)			
	44 − 3	42.30(20)			
Glu-γ-ha	011	9.50(1)			
	012	18.05(1)			
	013	20.26(1)			
	111	18.82(2)	14.42(2)	14.22(1)	18.92(5)
	110		7.84(2)	7.34(1)	
	212	33.24(2)			36.65(7)
	211	26.05(2)	20.51(5)	19.97(2)	31.70(7)
	210	17.00(3)	11.54(3)	10.90(2)	24.10(6)
	21 − 1	6.98(5)		0.48(3)	14.70(9)

* Ref. 7.

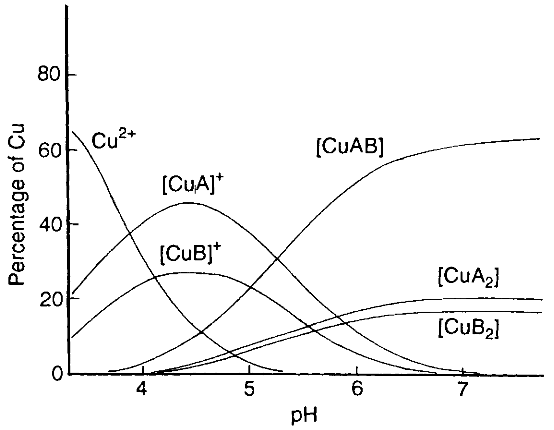


Fig. 3 Concentration distribution curves of complexes formed in copper(II)–aha-α-Ala system at 1:2:2 ratio, $c_{Cu^{II}}$ = 3 × 10^{-3} mol dm^{-3}

Tridentate co-ordination of Glu-γ-ha is possible only *via* joined five- and seven-membered chelates, while in the case of Asp-β-ha it occurs *via* the more stable joined five- and six-membered ones. The fact that the stability constants for the copper(II)–, nickel(II)– and zinc(II)–Glu-γ-ha complexes are higher than those for the simple amino acid or monohydroxamic acid models (*cf.* data in Table 1 and 2) supports the tridentate co-ordination of the Glu-γ-ha.

The different spectroscopic studies of the copper(II)–, nickel(II)– and zinc(II)–Glu-γ-systems give further support to co-ordination modes. Thus, the EPR parameters determined for the copper(II) Glu-γ-ha complexes [$g_{\parallel}$ = 2.24, $A_{\parallel}$ = 180 G (G = 10^{-4} T)] are in good agreement with those for copper(II)–simple amino acid species.[13] The visible spectra recorded for the same system show both amino-acid type (*N*,*O*) and hydroxamate type (*O*,*O*) chelates (the peak characteristic for hydroxamate co-ordination[14] appears above pH 5.5 and the colour of the sample turns to green).

The pH dependence of the spectra for the nickel(II)–Glu-γ-ha system in the region 300–800 nm is shown in Fig. 5.

The minimum value of λ_{max} assigned to the $^3A_{2g} \longrightarrow {}^3T_{1g}$ transition occurs at 631 nm and that of $^3A_{2g} \longrightarrow {}^3T_{1g}(P)$ at 371

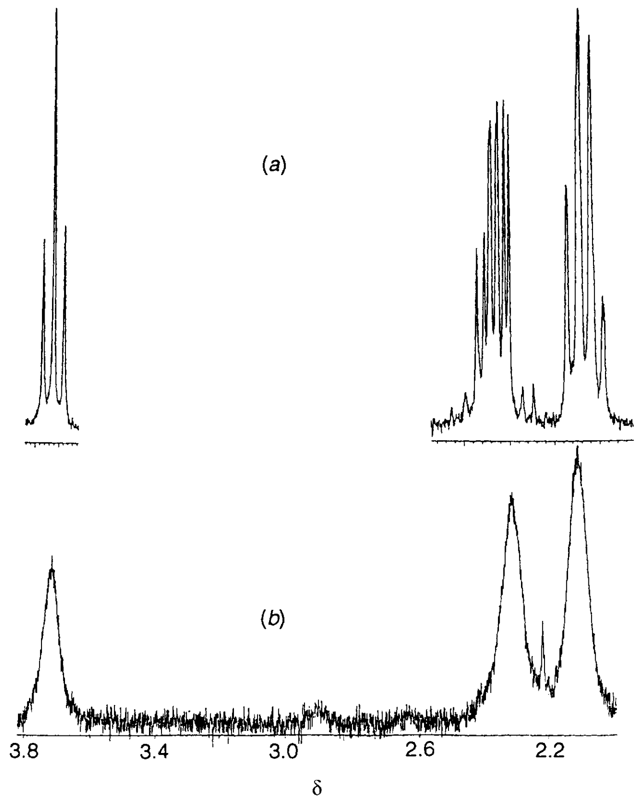


Fig. 4 Proton NMR spectra for Glu-γ-ha (*a*) and for Fe^{III}–Glu-γ-ha at 1 : 100 ratio (*b*)

Table 3 Antisymmetric C=O stretching bands (cm^{-1}) recorded for Asp-β-ha and Glu-γ-ha, at 0.05 mol dm^{-3} ligand concentrations, in D_2O

	Asp-β-ha		Glu-γ-ha	
Ligand[a]	Carboxyl	Hydroxamic	Carboxyl	Hydroxamic
H_3A^+	1734	1662	1729	1651
H_2A[b]	1625	1657	1619	1646
A^{2-}	1581	1618	1576	1614

[a] All labile protons are deuteriated. [b] CO_2^-, NH_3^+ and CONHOH are the functional groups present.

nm. These values indicate a lower number of *N*-donors in the co-ordination sphere than in nickel(II)-α-Ala (where in a sample with a 1 : 4 metal : ligand ratio, at pH *ca.* 10, the appropriate λ_{max} values are 600 and 365 nm, respectively), but higher than in the nickel(II)–aha system (λ_{max} 671 and 387 nm). The above results, together with the ^1H NMR line-broadening effects, show that co-ordination of Glu-γ-ha to the nickel(II) ion occurs from both ends of the molecule and involves formation of both types of chelates (*N*,*O*) and (*O*,*O*), probably in a dynamic equilibrium as shown in Scheme 3.

The same conclusion can be drawn from the IR results. Infrared measurements were made for Glu-γ-ha, Asp-β-ha and their nickel(II) and zinc(II) complexes (Table 3). The region (1800–1500 cm^{-1}) which contains the antisymmetric C=O stretching bands gives valuable information in aqueous (D_2O) solution.[14] The results for the ligands are summarized in Table 3.

These data are in good agreement with those published for the stretching bands of the carboxyl[15] and hydroxamic acid[16] groups. As found earlier, the co-ordination of the hydroxamate oxygens causes a shift of 35 cm^{-1} in the hydroxamate C=O band.[16] In contrast, co-ordination of the carboxylate to borderline metal ions does not cause any significant shift in the antisymmetric carboxylate C=O band.

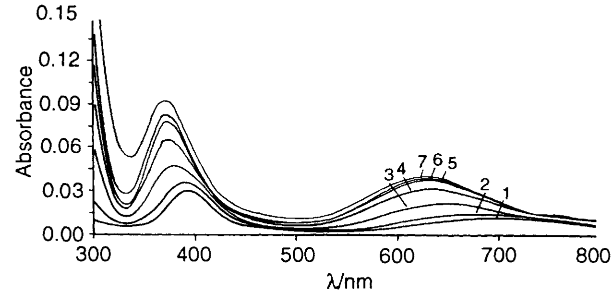


Fig. 5 The pH dependence of the spectra recorded for nickel(II)–Glu-γ-ha system at 1 : 4 metal : ligand ratio, $c_{Ni^{II}} = 2 \times 10^{-2}$ mol dm^{-3}; pH 4.25(1), 5.0(2), 6.0(3), 7.0(4), 8.0(5), 9.0(6) and 10.0(7)

According to our experimental results relating to metal ion–Glu-γ-ha systems, the stretching band of hydroxamic C=O remains at *ca.* 1615 cm^{-1} in all samples containing any ligand in excess, but without the ligand in excess it shifts and only a broad intense band occurs at *ca.* 1580 cm^{-1}.

Finally, ^1H NMR chemical shift measurements made on zinc(II)–Glu-γ-ha gave only *ca.* 0.01–0.02 ppm shifts from the free ligand at the same pH (pH $\leqslant$7.5), hydrolysis of the zinc(II) ion precluded measurements at higher pH values. Taking into account the results published recently on zinc(II)–aminohydroxamates (*e.g.* histidinehydroxamate, alaninehydroxamate),[17] namely, that chelation through hydroxamate oxygens does not cause any measurable chemical shift of CH proton signals, our results suggest that under the conditions used chelation occurs *via* the hydroxamate oxygens.

Conclusion

Co-ordination of Ni^{II}, Cu^{II}, Zn^{II} and Fe^{III} by Glu-γ-ha occurs by tridentate co-ordination with no evidence for polynuclear species under the conditions employed. For Fe^{III}, co-ordination involves the hydroxamate and carboxylate oxygen (*O*,*O*,*O*) mode only. For Ni^{II} and Cu^{II}, co-ordination occurs *via* the (*O*,*N*,*N*) and (*O*,*O*,*N*) modes [Scheme 2(*a*) and (*b*)], probably in dynamic equilibrium (Scheme 3). Finally, Zn^{II} is co-ordinated predominantly in the (*O*,*O*,*N*) mode [Scheme 2(*b*)].

Acknowledgements

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